

Understory algae and low light reduce recruitment of the habitat-forming kelp *Ecklonia radiata*

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ABSTRACT: Kelp forests form important habitat in temperate subtidal regions but are threatened by a range of stressors that impact their abundance. In this study, we determined the effect of understory algae, a potential competitor of kelp, on the recruitment of the Australasian kelp, *Ecklonia radiata*, via their role in reducing light availability for early life-cycle stages of *Ecklonia*. A field experiment manipulating the abundance of understory algae showed no recruitment of out-planted microscopic sporophytes or naturally recruiting macroscopic sporophytes with a high (>80%) cover of understory algae. In contrast, both microscopic and macroscopic sporophytes recruited into plots with a medium (40 to 60%) and low (<20%) cover of understory algae. Light appears to be the important mechanism for the inhibition of kelp recruitment because a high cover of understory algae blocks up to 90% of available light for *Ecklonia* recruits. A laboratory experiment showed that low light (similar to the levels beneath the understory of high-coverage algal canopies) inhibited *Ecklonia* gametophyte growth and prevented microscopic sporophyte recruitment. Both high light (similar to levels when there is little understory algal canopy) and high zoospore density resulted in high sporophyte recruitment, suggesting important additive effects of those factors. This study indicates that an increase in the cover of understory algae results in low light, which inhibits *Ecklonia* sporophyte recruitment. The resilience of kelp will be strongly influenced by recruitment, and our results highlight that a decline in kelp canopy cover which leads to an increase in understory algae can ultimately feedback to negatively affect kelp recruitment.

KEY WORDS: Laminariales · Gametophyte settlement · Sporophyte recruitment · Photosynthetically active radiation · PAR · Recruitment · Understory algae

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INTRODUCTION

Coastal marine ecosystems and their biodiversity are under threat from a range of anthropogenic stressors, including climate change, declining water quality and overfishing (Jackson et al. 2001, Airoldi 2003). In temperate coastal ecosystems, increasing ocean temperature impacts canopy-forming brown macroalgae, especially kelp (order Laminariales), reducing their growth, survivorship and density (Dayton et al. 1999, Graham et al. 2007, Staehr & Wernberg 2009, Wernberg et al. 2010, 2011, Johnson et al. 2011). Because kelp are foundation species and provide primary production and habitat for a diverse

assemblage of understory algae, invertebrates and vertebrates (Steneck et al. 2003, Graham et al. 2007, Steneck & Johnson 2014), a decline in their density has major consequences for coastal biodiversity.

Healthy kelp forests block light from reaching the benthos, often by up to 90% or more (Gerard 1984, Reed & Foster 1984, Kennelly 1989, Wernberg et al. 2005). Consequently, a reduced kelp density results in higher light beneath the canopy and an increase in the abundance of understory algae (Kennelly 1987a, Clark et al. 2004, Toohey et al. 2004, Flukes et al. 2014). A high abundance of fleshy and foliose understory algae can create a thick sub-canopy that reduces light to levels even lower than occur beneath

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the kelp canopy (Clark et al. 2004), which likely results in stronger competitive effects on small kelp recruits (Kennelly 1987b, Dayton et al. 1999, Clark et al. 2004, Connell & Russell 2010). While kelp gametophytes are able to survive at low light levels ($<5 \mu\text{mol photon m}^{-2} \text{s}^{-1}$) for several months, kelp sporophytes require higher light levels (>30 to $50 \mu\text{mol photon m}^{-2} \text{s}^{-1}$) for germination and growth (Novaczek 1984a, Kinlan et al. 2003, Carney & Edwards 2006). As well as a decrease in light, a high abundance of understory algae can also cause increased scour and sedimentation (Kennelly 1987a), which can also impact kelp recruitment (Geange et al. 2014). Thus, a decline in the kelp canopy cover results in changes to several abiotic factors that can negatively feedback to reduce kelp recruitment. In addition to these abiotic changes, a lower adult kelp density may also result in fewer zoospores, lower fertilization rates and reduced recruitment of kelp sporophytes (Reed 1990a,b, Reed et al. 1992). Although increasing light to the benthos because of kelp loss can result in a higher abundance of understory algae that compete with kelp recruits, the effects of the low light conditions created by understory algae on the survivorship and growth of critical early kelp life-cycle stages are not well understood. Moreover, the relative importance of abiotic (light intensity) and biotic (zoospore density) effects remains unclear.

In southern Australia, the dominant kelp is *Ecklonia radiata* (C. Agardh) J. Agardh (hereafter *Ecklonia*). *Ecklonia* is affected by increasing ocean temperature, with both microscopic and macroscopic recruitment, canopy cover and resilience all lower at higher temperatures (Wernberg et al. 2010, 2013, Mabin et al. 2013). Because a reduction in *Ecklonia* canopy cover results in an increase in the abundance of understory algae (Kennelly 1987a, 1989, Toohey et al. 2004, Flukes et al. 2014), understanding mechanisms behind competitive interactions between understory algae and the early life-cycle stages (gametophyte and juvenile sporophytes) of *Ecklonia* is important.

In this study, we determined the effect of increasing understory algae abundance on *Ecklonia* recruitment via its role in affecting light availability for juvenile stages of *Ecklonia*'s life-cycle. Specifically, we determined (1) the relationship between light availability beneath the *Ecklonia* canopy and understory algae biomass, (2) differences in light intensity beneath the understory algae compared to areas lacking understory algae, (3) the effect of understory algae coverage on the post-recruitment survivorship of microscopic and macroscopic *Ecklonia* sporo-

phytes, and (4) the effect of light intensity and zoospore density on the recruitment and growth of microscopic life-cycle stages of *Ecklonia*.

MATERIALS AND METHODS

Study species

Ecklonia radiata is the most widespread temperate marine habitat-forming species in southern Australia, occurring from near Geraldton in Western Australia across the entire southern coast of the mainland to near Caloundra in Queensland, as well as around Tasmania (Womersley 1967, Hatcher et al. 1987). Although *Ecklonia* has a depth range extending to ≥ 50 m, at our study location in Fortescue Bay, south-eastern Tasmania ($43^{\circ} 08' \text{S}$, $147^{\circ} 58' \text{E}$), *Ecklonia* forms extensive beds from a depth of ~ 6 m down to ≥ 15 m. The density of *Ecklonia* at this location is typically 8 to 12 adult sporophytes m^{-2} (Flukes et al. 2014). As at other locations (Kennelly 1989, Wernberg et al. 2005), light intensity beneath the *Ecklonia* canopy at Fortescue Bay is very low compared to light without a canopy ($\sim 95\%$ reduction; authors' unpubl. data).

Ecklonia has a typical kelp life cycle consisting of microscopic male and female gametophytes and a macroscopic sporophyte stage (Womersley 1981). Zoospores are released from sori on adult sporophytes and germinate into male and female gametophytes. Gametophytes are dioecious, with females larger (up to $400 \mu\text{m}$ end to end) and less branched than males (up to $100 \mu\text{m}$ end to end). Male gametophytes produce motile antherozoids that fertilise non-motile eggs which have been released from oogonia on females, giving rise to juvenile sporophytes (Womersley 1981). Adult sporophytes grow up to 2 m in height and form a dense canopy that blocks light for understory species (Wernberg et al. 2005). The reproductive season for *Ecklonia* varies with its distribution, but reproduction is typically higher in colder months of the year (Wernberg et al. 2010).

Relationships between light availability and understory algae biomass

We examined relationships between light availability and understory algae in 2 ways. First, we determined whether the amount of photosynthetically active radiation (PAR) beneath the *Ecklonia* canopy (but above the understory algal canopy) was

associated with the biomass of understory algae. At 3 sites (approximately 1 km apart from each other) in Fortescue Bay, twelve 0.25 m² quadrats were randomly placed at the depth of 10 m (± 1 m) where *Ecklonia* occurred. Adult *Ecklonia* density varied slightly among sites but averaged 8 sporophytes m⁻². Light intensity under the *Ecklonia* canopy was measured with an Odyssey handheld PAR light logger in the centre of each quadrat using SCUBA. All measurements were taken on the same day in May 2011. Accumulated photon density was recorded every 30 s for 2 min, resulting in 4 readings per quadrat. All understory algae, except for encrusting coralline algae, were collected and biomass determined by drying at 70°C for 48 h (Wright & Davis 2006). Variation in the biomass of understory algae as a function of light availability (mean photon flux density per quadrat; both square-root transformed to meet analysis of covariate [ANCOVA] assumptions) and site was determined using an ANCOVA with the categorical factor site (3 levels) and the continuous factor light (covariate). This analysis and all subsequent analyses were performed using R (v. 2.15.1).

Light availability beneath understory algae

Second, to understand the extent to which understory algae reduces light availability for microscopic *Ecklonia* life-cycle stages, we quantified PAR beneath understory algae compared to areas without understory algae. To do this, 2 Odyssey PAR logger were held on the substratum approximately 1 m apart. One sensor was placed beneath a haphazardly selected patch of understory algae, and the other was placed in an adjacent area at the same depth that lacked understory algae. Both readings were taken where there was no existing *Ecklonia* canopy and at least 2 m away from *Ecklonia* plants to avoid edge effects from surrounding canopies. Readings were taken simultaneously in both places every second for between 90 and 150 s for each pair. Integrated (average) light intensities for 10 s blocks of time were determined, and the mean of these values over the entire time period for each replicate (range between 9 and 15 measurements per replicate) was compared between areas with and without understory algae using a paired *t*-test. There were $n = 5$ replicate paired readings taken, with all readings done within a 20 min period between 12:50 and 13:10 h on a mostly sunny day in April. Thus, we have determined the relative reduction in light due to the understory algae canopy on one day.

Effect of understory algae cover on post-recruitment survivorship of *Ecklonia*

Manipulation of understory algae

To determine the effects of understory algae on *Ecklonia* recruitment, an experiment was conducted at Fortescue Bay between March and August 2012. Twelve 0.25 m² quadrats were established along a 30 m transect on a relatively flat reef at a depth of 10 m (± 1 m) within an *Ecklonia* forest. Quadrats were marked at the corners with underwater epoxy (A-788 Splash Zone Compound). Understory algae within each quadrat were removed by hand to establish 3 treatments: low (<20% cover), medium (40 to 60% cover) and high (>80% cover; unmanipulated) understory algae ($n = 4$ quadrats per treatment). The percentage cover of understory algae was determined using a gridded quadrat (each cell represented 4% cover). Quadrats were separated by at least 1.5 m to avoid overlap, and a 50 cm buffer zone was trimmed around each quadrat of the same cover of understory algae. The *Ecklonia* canopy within and around quadrats was trimmed to isolate the effect of the understory on *Ecklonia* recruitment. All macroscopic *Ecklonia* juveniles (<27 cm length, Kirkman 1984) were also removed at the start of the experiment but not thereafter. Quadrats were maintained for 5 mo with understory algae weeded as required every month to maintain the treatments within the required ranges.

Culturing of microscopic sporophytes

Microscopic sporophytes were cultured in the lab for outplanting into experimental plots. Spores were released from 12 *Ecklonia* sporophytes collected from a depth of 10 m at Fortescue Bay. These were held overnight in aerated sea water at 17°C, then sori from each thallus were cut into approximately 5 × 5 cm pieces, dried with paper towel and placed into a pre-sterilised container in a dark room at 17°C for approximately 1 h. After 1 h, the sori were washed in Betadine solution (1 ml l⁻¹ distilled H₂O) for 1 min to remove bacteria, and any excess mucus was wiped off with a lint-free cloth. The clean sori were placed into a different pre-sterilised container filled with 500 ml of standard f/2 sea water media (Anderson 2005). The container was kept at 17°C under a constant light (133 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$) for 1 h to release zoospores. The density of zoospores in the solution was determined by a haemocytometer to be 1 040 000 zoospores ml⁻¹. The average number of zoospores

contained in 1 mm² of sori in the high reproductive season for *Ecklonia* is approximately 2500 (Mohring et al. 2012); therefore, using undiluted zoospore solution would result in an unnaturally high zoospore density. Zoospores were released onto sterile 7.84 cm² ceramic tiles (n = 70) placed individually into 70 ml jars at a more realistic concentration of approximately 7200 zoospores ml⁻¹. Twelve tiles without spores were also established as controls. Tiles were kept at 17°C under a 16 h light: 8 h dark cycle at >100 μmol photon m⁻² s⁻¹ and kept in culture for 35 d, by which time sporophytes were growing (Mabin et al. 2013). Approximately 80% of the media was changed once half way through the culturing period.

Out-planting and natural recruitment

After 35 d, the number of sporophytes on each tile was counted under a dissecting microscope before out-planting. Tiles with no visible sporophytes were discarded (38 tiles were not used). This absence of sporophytes in some tiles appeared to be due to bacterial contamination of some culture jars. The control tiles were also assessed, and none had any sporophytes on them. Each tile was randomly allocated to 1 of the 3 understory algal treatments, with each quadrat receiving 2 to 3 sporophyte tiles plus 1 control tile to assess whether any recruitment occurred on tiles post-outplanting. The initial sporophyte number on each tile was determined, and tiles were transported to Fortescue Bay in their jar in a cooler and then taken underwater. The tiles were carefully removed from jars underwater and attached to the rock substrate within quadrats using underwater epoxy (A-788 Splash Zone Compound). The tiles were left in the quadrats for 4 wk, then collected by gently prising them from the rock, and placed into jars before being taken back to the lab. The tiles were assessed for any remaining *Ecklonia* sporophytes under a dissecting microscope. No sporophytes were found on the control tiles in any of the treatments. The number of naturally occurring *Ecklonia* sporophytes within each quadrat was counted visually at the same time the tiles were collected.

Differences in the abundance of out-planted sporophytes and natural sporophyte recruitment among understory algae coverage treatments were analysed using generalised linear models (GLM-multicomp package, R v. 2.15.1) in an analysis of deviance framework because these data had many zeros. GLMs were performed with Poisson (out-planted sporophytes) or quasi-Poisson (natural recruitment)

distributions depending on the degree of data dispersion (determined following Zuur et al. 2009) with a log-link. The abundance of out-planted sporophytes was compared among understory algae cover treatments, with quadrats nested within cover treatments and initial sporophyte density on tiles (pre-out-planting) as a continuous covariate. Natural sporophyte recruitment was compared among understory algae cover treatments only. Significance was tested against a χ^2 distribution, which is appropriate for a Poisson distribution (Zuur et al. 2009). Multiple comparison tests among different algal understory treatments were determined using the multicomp package in R v. 2.15.1 (Hothorn et al. 2008).

Effect of light intensity and zoospore density on recruitment and growth

Experimental conditions

Spore release and general culture methods (culture media, jars, temperature and light cycle) were the same as for the experiment to determine the effect of understory algae on microscopic sporophyte recruitment except spores were released onto three 14 mm diameter glass coverslips per jar instead of ceramic tiles.

To determine the interactive effects of light and zoospore density on recruitment, 3 light intensity treatments were crossed with 3 zoospore density treatments. These different conditions were established in 90 jars (n = 10 per treatment combination) containing 50 ml UV-sterilised enriched sea water media. To make the 3 different zoospore density treatments, dilutions of a stock solution were placed into the jars with the coverslips. The 3 initial zoospore densities were ~7200 ml⁻¹ (high), ~3600 ml⁻¹ (medium) and ~2400 ml⁻¹ (low). Three different light levels were created by covering the jars with layers of mesh to meet designated light levels of >100 (high light), 40 to 60 (medium light) and <10 (low light) μmol photon m⁻² s⁻¹. These light intensities were chosen because they cover the natural range in PAR recorded beneath understory algae (low light treatment), up to that recorded without understory algae (high light treatment; see 'Results') within *Ecklonia* forests at our site. Similar light levels have been used in previous experiments with *Ecklonia* (Novaczek 1984a). Light intensity in jars was measured during the experiment and ranged from 166 to 233 μmol photon m⁻² s⁻¹ (high light), 40 to 58 μmol photon m⁻² s⁻¹ (medium light) and 3 to 7 μmol photon m⁻² s⁻¹ (low light).

Gametophyte and sporophyte assessment

After 14 d, 1 of the 3 coverslips in each jar was removed to determine the density and size of male and female gametophytes. Coverslips were placed upside down on a glass slide to ensure any growing gametophytes were flat so 2-dimensional surface area could be measured. Twelve photographs were randomly taken of each coverslip under 40 \times magnification. Three images were then randomly chosen from those 12, and each photo was then further divided into 12 sections using the grid maker plug-in (each section was 0.7 mm²) within Image J 1.45s. Five of those sections were randomly chosen from each image, and all male and female gametophytes in those sections were counted to determine their density (gametophytes per 3.5 mm²). An additional 20 photographs (magnification = 200 \times) were randomly taken, and 1 male and female gametophyte each were randomly selected from 5 of those photographs and their size measured based on surface area determined using Image J. Differences in the abundance and size of male and female gametophytes were determined separately using 3 factor nested ANOVAs with the factors light intensity (fixed) and initial zoospore density (fixed) with jars (random) nested within the light \times zoospore density interaction. Following ANOVAs, Tukey tests were performed separately within each treatment combination.

A similar assessment was conducted on Day 30 to determine the density and size of sporophytes. The number of sporophytes on each coverslip was counted directly under 40 \times magnifications. All sporophytes on each coverslip were counted. To determine sporophyte size, 5 to 12 random images were taken on each coverslip (magnification = 100 \times), and 5 sporophytes per replicate were randomly chosen and 2-dimensional surface area determined with Image J. A 2-factor ANOVA with the factors light intensity and initial zoospore density was used to determine differences in sporophyte density, while a 3-factor nested ANOVA with the factors light intensity, initial zoospore density and jars nested within the light \times zoospore density interaction was used to determine differences in sporophyte size. For the analyses of sporophyte density and size, only medium and high light treatments were included because no sporophytes developed at low light. A further 12 jars were excluded from the analyses because of contamination. Following the ANOVAs, Tukey post-hoc tests were done.

RESULTS

Relationships between light intensity and understory algae

Light intensity beneath the *Ecklonia* canopy was typically <100 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$, and there was a significant positive relationship between light intensity and understory algal biomass ($F_{1,32} = 12.914$, $p = 0.001$; Fig. 1A). Light intensity also varied among sites ($F_{2,32} = 10.284$, $p < 0.001$). Light intensity beneath the understory algae canopy was typically very low (<10 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$), and there was significantly less light beneath the understory ($12.4 \pm 4.0 \mu\text{mol photon m}^{-2} \text{s}^{-1}$, mean \pm SE) than at sites without understory ($95.8 \pm 1.9 \mu\text{mol photon m}^{-2} \text{s}^{-1}$, mean \pm SE, $t_5 = 15.627$, $p < 0.001$; Fig. 1B).

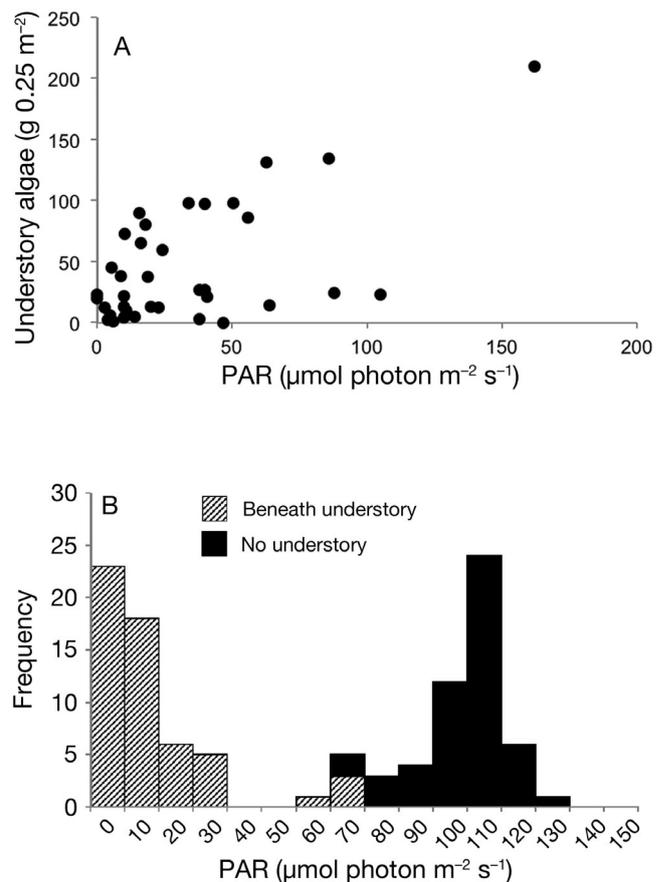


Fig. 1. (A) The relationship between photosynthetically active radiation (PAR) under the *Ecklonia radiata* canopy and the biomass of understory algae, and (B) frequency histogram of PAR measured beneath the understory algal canopy and without understory algae

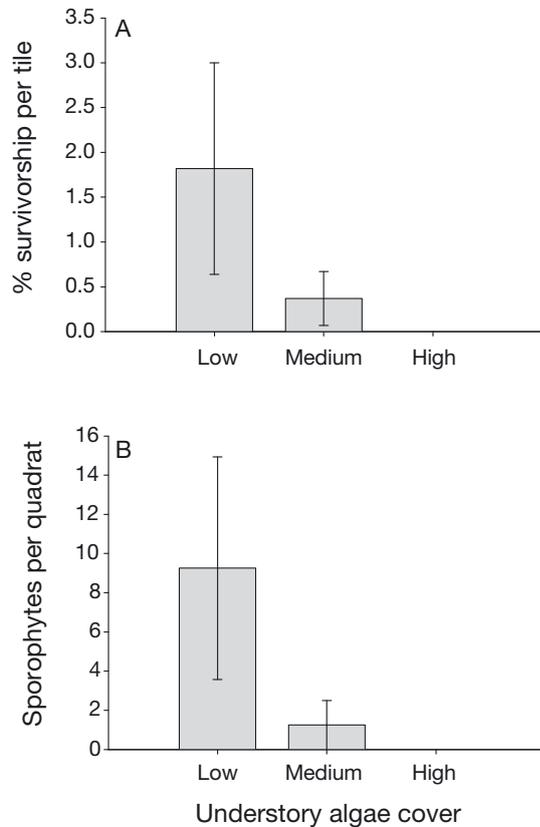


Fig. 2. Mean (± 1 SE) (A) percentage survivorship of outplanted microscopic *Ecklonia radiata* sporophytes remaining per tile after 28 d in the field and (B) number of naturally recruiting sporophytes into 0.25 m² quadrats of low (<20%), medium (40–60%) and high (>80%) understory algae cover

Effect of understory algae cover on post-recruitment survivorship of *Ecklonia*

There was no successful sporophyte recruitment (both outplanted microscopic and natural macroscopic) into the high understory algal cover treatment (Fig. 2). GLMs indicated a significant difference among treatments for both types of recruitment (Table 1), and post hoc tests showed that low and medium understory algal covers had higher densities of both microscopic and macroscopic sporophyte recruits compared to the high understory algal cover (both $p < 0.001$) with no difference between the low and medium understory treatments (Table 1, Fig. 2). The initial number of sporophytes present on outplanted tiles was a significant predictor of microscopic recruitment after 4 wk (Table 1). The outplanted sporophytes grew during the 4 wk of transplantation from 0.43 ± 0.07 mm² (mean \pm SE) before outplanting to 3.04 mm² \pm 1.08 (mean \pm SE)

Table 1. Analyses of deviance examining the effects of understory algae coverage treatment, quadrat within understory algae coverage treatment and initial *Ecklonia radiata* sporophyte density on out-planted microscopic sporophyte recruitment and understory algae coverage treatment on natural macroscopic *Ecklonia* sporophyte recruitment. Res.: residual

Factor	df	Deviance	Res. df	Res. deviance	p (>F)
Microscopic recruitment (Poisson fit)					
Null	30	48.625			
Understory algae	2	12.1242	28	36.501	0.002
Quadrat (Understory)	9	19.124	18	11.363	0.024
Initial density	1	6.014	27	30.486	0.014
Natural recruitment (quasi-Poisson fit)					
Null	11	120.63			
Understory algae	2	61.621	9	59.009	0.008

after outplanting (pooled across medium and low understory algal coverage treatments, measured using Image J).

Effect of light intensity and zoospore density on recruitment and growth

Gametophytes

The abundance of both male and female gametophytes decreased with increasing light intensity (Table 2, Fig. 3A,B). Male gametophyte abundance was reduced by approximately a third in medium light and by half in high light compared to the low-light treatment (Table 2, Fig. 3A). Female gametophyte abundance was reduced by approximately half in high light compared to the medium- and low-light treatments, which had similar densities (Table 2, Fig. 3B). High initial zoospore densities resulted in significantly more male and female gametophytes compared to medium and low zoospore densities (Table 2, Fig. 3A,B), approximately double that of the other 2 treatments for both sexes. For both male and female gametophyte abundance, there was no interaction between light and zoospore density, but there was significant variation among jars within the light \times zoospore density interaction (Table 1).

Both male and female gametophyte size varied with light and zoospore density (significant light \times zoospore density interaction; Table 2). Tukey's tests within each light treatment showed that male gametophytes were larger at high initial zoospore density compared to medium and low zoospore density treatments in high light; were the same size in all zoo-

Table 2. ANOVAs testing for differences in the density and size of male and female *Ecklonia radiata* gametophytes under different light and initial zoospore density treatments with replicate jars nested within the light \times zoospore interaction. Density data were square root transformed, and the size data were \log_{10} transformed to meet ANOVA assumptions. Results of Tukey's tests testing for differences between light and initial zoospore density treatments (gametophyte density) or done within the light \times zoospore density interaction (gametophyte size) are shown. Abbreviations: low light (LL), medium light (ML), high light (HL); high zoospore density (HZ), medium zoospore density (MZ) and low zoospore density (LZ)

Dependent variable	Source	df	SS	MS	F	p (>F)
Male density	Light	2	193.197	96.589	62.275	<0.001
	Zoospore	2	180.651	90.325	58.237	<0.001
	Light \times Zoospore	4	9.643	2.411	1.554	0.195
	Jar(Light \times Zoospore)	80	124.050	1.551	6.863	<0.001
	Residuals	178	40.264	0.226		
Tukey's tests: Light: LL > ML > HL Zoospore: HZ > MZ = LZ						
Female density	Light	2	271.661	135.831	66.356	<0.001
	Zoospore	2	238.368	119.184	58.224	<0.001
	Light \times Zoospore	4	12.718	3.179	1.553	0.195
	Jar(Light \times Zoospore)	80	163.776	2.047	5.444	<0.001
	Residuals	178	66.852	0.376		
Tukey's tests: Light: LL = ML > HL Zoospore: HZ > MZ = LZ						
Male size	Light	2	8.866	4.433	43.375	<0.001
	Zoospore	2	0.491	0.246	2.403	0.001
	Light \times Zoospore	4	2.791	0.698	6.827	<0.001
	Jar(Light \times Zoospore)	80	8.174	0.102	4.189	<0.001
	Residuals	352	8.606	0.024		
Tukey's tests: High Light: HZ > MZ = LZ, Medium Light: HZ = MZ = LZ, Low Light: LZ > MZ = HZ High Zoospore: HL = ML > LL, Medium Zoospore: ML > HL > LL, Low Zoospore: HL = ML = LL						
Female size	Light	2	16.062	8.031	51.579	<0.001
	Zoospore	2	0.336	0.168	1.079	0.004
	Light \times Zoospore	4	2.135	0.534	3.428	<0.001
	Jar(Light \times Zoospore)	81	12.613	0.156	5.561	<0.001
	Residuals	359	10.043	0.028		
Tukey's tests: High Light: HZ > MZ = LZ, Medium Light: HZ = MZ = LZ, Low Light: LZ > MZ = HZ High Zoospore: HL = ML > LL, Medium Zoospore: HL = ML > LL, Low Zoospore: ML > HL = LL						

spore density treatments in medium light; and were larger at low initial zoospore density compared to high and medium zoospore density treatments in low light (Table 2, Fig. 3C). Within the high zoospore density treatment, male gametophytes were larger at high and medium light compared to the male gametophytes at low light, which were ~25 to 50% smaller in size; within the medium zoospore density treatment, male gametophytes were larger at medium light compared to high light (~50% larger in size) and both were larger than in low light (~50% larger in size) (Table 2, Fig. 3C); within the low zoospore density treatment, male gametophytes were the same size in all light treatments.

The response of female gametophyte size was similar to males. Within the high-light treatment,

females were largest at a high zoospore density; within the low-light treatment, females were largest at high and medium zoospore density; and within the medium-light treatment, there was no difference in size among zoospore density treatments (Table 2, Fig. 3D). Within the high and medium zoospore density treatments, female gametophytes were significantly smaller at low light compared to high and medium light treatments (~30 to 70% smaller in size; Fig. 3D). At low zoospore density, female gametophytes were significantly smaller at high and low light compared to the medium light treatment.

Morphologically, gametophytes at high and medium light often had thick branching filaments, whereas gametophytes at low light had thin and long filaments (authors' pers. obs.). Female gametophytes in high

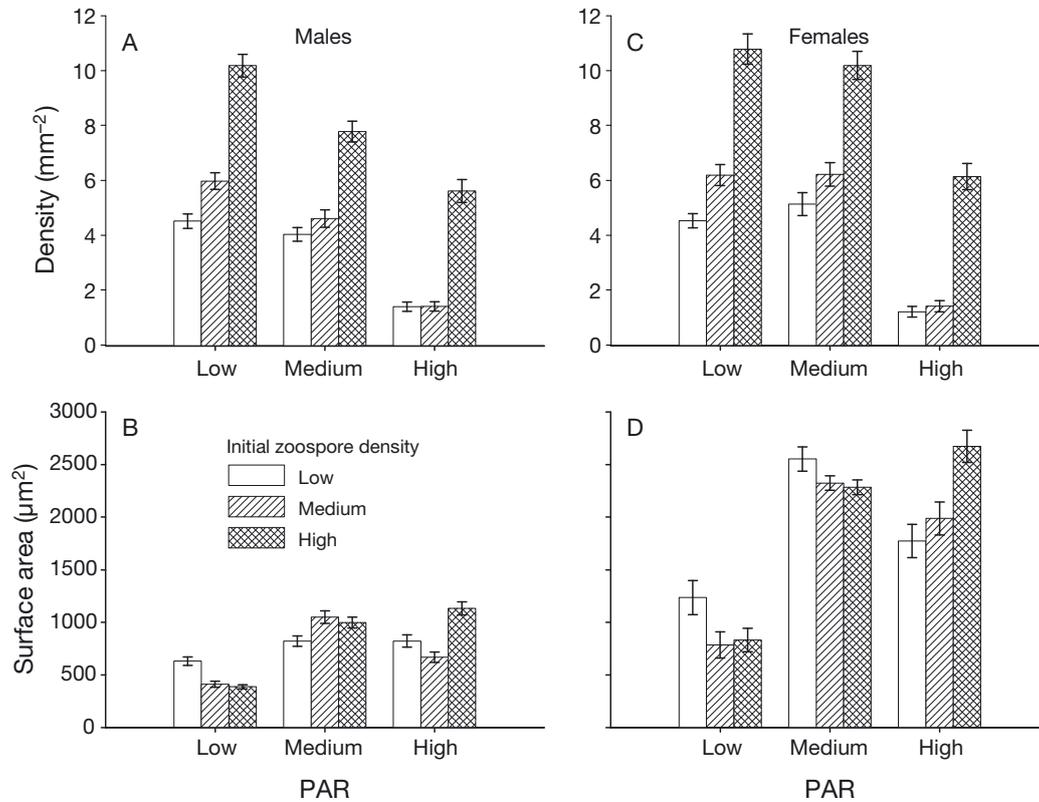


Fig. 3. Mean (± 1 SE) (A,C) density and (B,D) surface area of (A,B) male and (C,D) female gametophytes of *Ecklonia radiata* after 14 d when grown under low, medium and high PAR and with low, medium and high initial zoospore density. See Table 2 for results of Tukey's tests

and medium light treatments also appeared to have larger cells, and we observed eggs being released from oogonia. No such development was observed in the low-light treatment.

Sporophytes

No sporophytes developed at low light, and the highest number of sporophytes occurred when there was both high light and high initial zoospore density (significant light \times initial zoospore density interaction; Table 3, Fig. 4A). Tukey's tests done within light treatments showed that in high light, there were more sporophytes at high zoospore density compared to both medium and low zoospore density, but in medium light, sporophyte densities were the same in all zoospore density treatments. Tukey's tests done within each zoospore density treatment showed more sporophytes in high light than medium light for the high zoospore density treatment but similar sporophyte densities across light treatments for the medium and low zoospore density treatments (Table 3, Fig. 4A).

The effect of light on sporophyte size depended on initial zoospore density (significant light \times zoospore density interaction), and there was significant variation among the jars within treatments (Table 3). Within the high-light treatment, there was no difference among zoospore densities, while within the medium-light treatment, sporophytes were larger at a high zoospore density compared to medium and low zoospore density treatments (Table 3). Within the high zoospore density treatment, sporophytes were larger in medium light compared to high light, but there were no differences in sporophyte size at high and medium light within the medium and low zoospore density treatments (Table 3, Fig 4B).

DISCUSSION

We have demonstrated that a high cover of understory algae inhibits *Ecklonia* sporophyte recruitment and that low light intensity beneath understory algal canopies is likely to be a major mechanism for this inhibition. Although low light resulted in higher recruitment of gametophytes in the lab experiment,

Table 3. ANOVA testing for the differences in number and size (size \log_{10} transformed to meet ANOVA assumptions) of *Ecklonia radiata* sporophytes under different light and initial zoospore density treatments. Only medium and high light treatments were included because no sporophytes developed at low light. Results of Tukey's tests done within the light \times zoospore density interactions are shown. Abbreviations: low light (LL), medium light (ML), high light (HL); high zoospore density (HZ), medium zoospore density (MZ) and low zoospore density (LZ)

Dependent variable	Source	df	SS	MS	F	p (>F)
Density	Light	1	11476	11476	0.571	0.454
	Zoospore	2	64046	32023	1.593	0.215
	Light \times Zoospore	2	198942	99471	4.950	0.012
	Residuals	42	844078	20097		
Tukey's tests:						
High Light: HZ > MZ = LZ, Medium Light: HZ = MZ = LZ						
High Zoospore: HL > ML, Medium Zoospore: HL = ML, Low Zoospore: HL = ML						
Size	Light	1	0.699	0.699	3.152	0.083
	Zoospore	2	2.644	1.322	5.959	0.005
	Light \times Zoospore	2	1.312	0.656	2.957	0.026
	Jar(Light \times Zoospore)	43	9.540	0.222	1.523	0.029
	Residuals	196	28.544	0.146		
Tukey's tests:						
High Light: HZ = MZ = LZ, Medium Light: HZ > MZ = LZ						
High Zoospore: ML > HL = LL, Medium Zoospore: HL = ML = LL, Low Zoospore: HL = ML = LL						

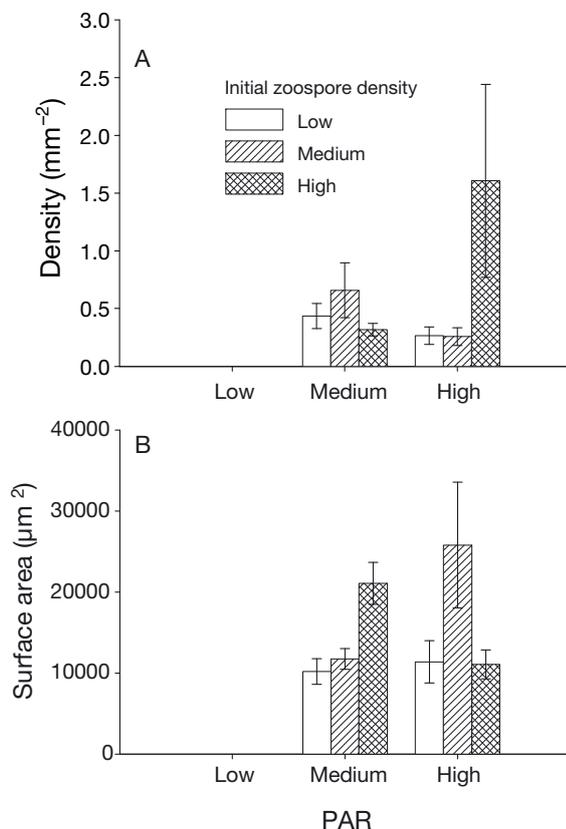


Fig. 4. Mean (± 1 SE) (A) density and (B) surface area of sporophytes of *Ecklonia radiata* after 30 d when grown under low, medium and high PAR and with low, medium and high initial zoospore density. See Table 3 for results of Tukey's tests

these gametophytes grew poorly, and there was no successful recruitment of sporophytes under low light. Overall, these findings indicate that a reduction in the *Ecklonia* canopy cover can indirectly lead to lower *Ecklonia* recruitment because understory algae increase in abundance and create low-light conditions that inhibit sporophyte recruits.

Light intensity and understory algae biomass were positively associated, in line with a number of previous studies showing that an increase in light following the removal of *Ecklonia* canopy results in an increase in the abundance of understory algae (Kennelly 1989, Toohy et al. 2007, Flukes et al. 2014). The understory algae community in southeastern Tasmania is diverse and fleshy, and foliose species make a large proportion of the understory cover (Edgar et al. 2004, Flukes et al. 2014). As we observed in the lab experiment, high light is a crucial resource required for the germination and growth of *Ecklonia* sporophytes (also see Kirkman 1981). Under a full understory algae canopy, light levels were typically $< 10 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$, which was the light level when no sporophytes developed in the lab. Although we only measured light beneath the understory on one day, our measurements demonstrate the relative amount to which understory algae blocks light to the benthos (up to 90%). Importantly, no outplanted microscopic sporophytes survived under a high understory algae cover. Similarly, in Californian kelp forests, a high cover of the understory brown alga *Desmarestia ligulata* reduces light to the ben-

thos by up to 99% compared to areas lacking *Desmarestia* (Clark et al. 2004), which limits *Macrocystis pyrifera* recruitment (Dayton et al. 1999). Our results also suggest large fluctuations in light that occur over small temporal scales (seconds) as the understory canopy moves with waves. It is likely that beneath a moderate understory algal cover, light levels will fluctuate between ~ 10 and $100 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ because of movement of the understory and *Ecklonia* canopies. Even under a full understory canopy, we observed light levels as high as $70 \mu\text{mol photon m}^{-2} \text{s}^{-1}$. In our field experiment, an understory algal cover between 40 and 60% resulted in similar post-recruitment survivorship and natural recruitment to when there was <20% cover, suggesting that light above a certain threshold, or fluctuating light due to canopy movement, may be sufficient for successful *Ecklonia* recruitment.

The higher natural recruitment we observed at medium and low understory algal cover may be due to the growth of pre-existing microscopic recruits (gametophytes or sporophytes) in plots at the time of the manipulations or new zoospores that recruited into plots after the manipulations. A pulse of *Ecklonia* recruitment is often observed following the removal of an *Ecklonia* canopy (Kirkman 1981, Kennelly 1987b, Flukes et al. 2014), although it is not clear whether these are recruits present as microscopic stages prior to canopy removal or recruited afterward. Microscopic stages of kelp, especially gametophytes, can delay growth or development under low light or nutrients (Kinlan et al. 2003, Carney & Edwards 2006). If microscopic *Ecklonia* gametophytes or sporophytes are already present at the time of canopy loss, rapid post-recruitment growth may allow *Ecklonia* recruits to grow above the understory algae and outcompete them (Kennelly 1987a). The survivorship of out-planted sporophytes (35 d old at transplantation) for 4 wk under medium and low understory algae cover indicates new recruits could grow and survive in those treatments during the ~ 5 mo of the manipulations.

Although we have shown that understory algae reduce light availability, which inhibits kelp recruitment, understory algae are also likely to cause other abiotic changes, which may also negatively affect kelp recruitment. Gametophytes and early stage of kelp sporophytes are susceptible to scour and sedimentation. Scour from understory algae may remove gametophytes and sporophytes via a whiplash effect (Kennelly 1989), while sediment load and regime reduces zoospore establishment (Devinny & Volsse 1978, Geange et al. 2014). There may also be inter-

active effects between different factors. For example, the effects of sediment on furoid germlings are reduced in high light because the germlings have a faster growth rate (Irving et al. 2009). In addition to abiotic factors, grazers consume kelp microscopic stages at both pre- and post-settlement (Henríquez et al. 2011, VanMeter & Edwards 2013). Although these factors were not measured in this study, a higher cover of understory algae may potentially increase levels of these stressors. Overall survivorship of the outplanted microscopic *Ecklonia* sporophytes was low (0.64%), which is consistent with other kelp for which early post-recruitment mortality has been measured (Schiel & Foster 2006), and this pattern highlights the range of potential factors that can affect the survivorship of microscopic life-cycle stages of kelp.

Male and female *Ecklonia* gametophyte densities were highest under low light. Kelp, including *Ecklonia*, are often more fecund during autumn and winter (Mohring et al. 2013), and kelp gametophytes are resilient to low light and able to survive under such conditions for several months (Novaczek 1984b, Kinlan et al. 2003, Carney & Edwards 2006). A high light intensity can be fatal to kelp gametophytes (Novaczek 1984b, Wiencke et al. 2007), although our high light intensity treatment (166 to $233 \mu\text{mol photon m}^{-2} \text{s}^{-1}$) was well below the previously described fatal light intensity for *Ecklonia* gametophytes ($1000 \mu\text{mol m}^{-2} \text{s}^{-1}$; Novaczek 1984a). Light levels measured during autumn at our site, which was at 10 m depth, rarely exceeded $100 \mu\text{mol photon m}^{-2} \text{s}^{-1}$, although a reading of $162 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ was recorded. However, light can be significantly higher during summer (up to $394 \mu\text{mol photon m}^{-2} \text{s}^{-1}$; M. Tatsumi unpubl. data). Zoospore settlement in *Pterygophora californica* was also lower under high light, but zoospore settlement of *Macrocystis pyrifera* was not (Cie & Edwards 2008). However, high light appears to result in reduced germ tube growth and sporophyte production in *Macrocystis* (Cie & Edwards 2008). Not surprisingly, a high initial zoospore density resulted in higher recruitment of *Ecklonia* gametophytes compared to both medium and low initial zoospore density. The medium and low initial zoospore treatments only differed by approximately $1200 \text{ zoospore ml}^{-1}$, which may explain why there was no significant difference in the number of gametophytes between those 2 treatments. Overall, for *Ecklonia*, it appeared that high gametophyte recruitment is strongly dependent on the density of zoospores that are released and a low-to-medium light intensity. Such conditions should occur when

there is a canopy of *Ecklonia* or moderate cover of understory algae.

In contrast to the positive effects of low light intensity on gametophyte density, low light inhibited gametophyte growth, particularly at medium and high zoospore densities. The lower growth at low light may indicate a plastic vegetative 'guerrilla' growth form in *Ecklonia* gametophytes which can occur in algae under light-limited conditions (Monro & Poore 2005). Similar to our findings, Novaczek (1984a) also found that *Ecklonia* gametophytes developed long, thin non-reproductive filaments in lower light conditions ($<40 \mu\text{mol m}^{-2} \text{s}^{-1}$). Thus, under low light, *Ecklonia* gametophytes may germinate but delay growth and/or development until light intensity increases. In the field, fluctuating light intensity due to movement of the canopy, gaps opening up due to disturbance, season or water turbidity (Kennelly 1989, Reed 1990a, Toohey et al. 2004) may create windows for gametophyte growth and subsequent sporophyte recruitment. The larger size of both males and female gametophytes at high zoospore density and high light intensity suggests positive density-dependence at high light (Schiel & Choat 1980).

Significantly, there was no sporophyte recruitment under low light regardless of the initial density of zoospores. This absence is presumably linked to the poor growth of gametophytes, the absence of fertile oogonia or viable antherozoids in gametophytes at low light or post-fertilisation mortality. Novaczek (1984a) also noted that reproduction in female *Ecklonia* gametophytes required light conditions above 10 to $20 \mu\text{mol m}^{-2} \text{s}^{-1}$. Thus, initial positive effects of low light on gametophyte recruitment did not continue through their development. The highest sporophyte recruitment occurred under high light and high initial zoospore density, highlighting important additive effects of those factors. Coupled with the finding that outplanted sporophyte survivorship in the field was influenced by the initial number of sporophytes on tiles, it appears that both a high abundance of spores and the presence of adequate light are essential for successful sporophyte recruitment. High densities of gametophytes will increase the probability of fertilisation of oogonia by antherozoids and thus sporophyte densities. Similar positive effects of zoospore density on sporophyte recruitment have been shown for *M. pyrifera* and *P. californica* (Reed 1990b). Interestingly, most light intensities measured under the *Ecklonia* canopy in the field were less than the levels in the high light treatment in the lab, reinforcing the importance of gaps in the canopy creating high light

conditions for *Ecklonia* sporophyte recruitment (Toohey et al. 2007). Sporophyte size also varied with light and initial zoospore density but was not necessarily larger under high light. The reason for this finding was unclear, and the result was not consistent with Novaczek (1984a), who found that the growth of sporophytes increased with increasing light.

Our findings that low light has positive effects on gametophyte density but negative effects on gametophyte growth and sporophyte density reinforces the importance of an intact *Ecklonia* canopy for zoospore settlement but gaps in the canopy for subsequent sporophyte growth. As well as providing low-light conditions, the presence of an intact *Ecklonia* canopy will also affect the density of zoospores available to recruit. The intact canopy will contain sexually mature adult sporophytes, and although per capita zoospore production may be negatively correlated with adult kelp density due to negative density-dependence, the total density of zoospores in an area should increase with the density of adult sporophytes (Reed 1990a).

Overall, this study indicates that a high cover of understory algae inhibits *Ecklonia* sporophyte recruitment, and the most likely mechanism is a reduction in light, needed for growth and development. Although much work is still to be done, the implication of our study is that the engineering of the abiotic (light) and biotic (understory algae) environment by *Ecklonia* can create conditions that facilitate its successful recruitment resulting in a positive 'environment-engineer' feedback (Jones et al. 2010).

Acknowledgements. We thank Ian Jermyn, Martin Filleul and Emma Flukes for help in the field and Chris Mabin for assistance with the lab culturing.

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Editorial responsibility: Lisandro Benedetti-Cecchi, Pisa, Italy

*Submitted: April 27, 2015; Accepted: April 22, 2016
Proofs received from author(s): June 9, 2016*